

We claim:

1. An improved method for simultaneous sequence-specific identification of mRNAs in an mRNA population comprising the steps of:
 - (a) preparing a double-stranded cDNA population from an mRNA population
 - 5 using a mixture of anchor primers, each anchor primer having a 5' terminus and a 3' terminus and including: (i) a tract of from 7 to 40 T residues; (ii) a site for cleavage by a first restriction endonuclease that recognizes more than six bases, the site for cleavage being located towards the 5'-terminus relative to the tract of T residues; (iii) a first stuffer segment of from 4 to 40 nucleotides, the first stuffer segment being located towards the
 - 10 5'-terminus relative to the site for cleavage by the first restriction endonuclease; (iv) a second stuffer segment interposed between the site for cleavage by a first restriction endonuclease that recognizes more than six bases and the tract of T residues, and (v) phasing residues located at the 3' terminus of each of the anchor primers selected from the group consisting of -V, -V-N, and -V-N-N, wherein V is a deoxyribonucleotide selected
 - 15 from the group consisting of A, C, and G; and N is a deoxyribonucleotide selected from the group consisting of A, C, G, and T, the mixture including anchor primers containing all possibilities for V and N;
 - (b) cleaving the double-stranded cDNA population with the first restriction endonuclease and a second restriction endonuclease, the second restriction endonuclease
 - 20 recognizing a four-nucleotide sequence, to form a population of double-stranded cDNA molecules having first and second termini, respectively;
 - (c) inserting each double-stranded cDNA molecule from step (b) into a vector in an orientation that is antisense with respect to a bacteriophage-specific promoter within the vector to form a population of constructs containing the inserted cDNA molecules,
 - 25 thereby defining 5' and 3' flanking vector sequences adjacent to the 5' terminus of the sense strand of the inserted cDNA and the 3' terminus of the sense strand respectively, and said constructs having a 3' flanking vector sequence at least 15 nucleotides in length between said first restriction endonuclease site and a site defining transcription initiation in said promoter;
 - (d) transforming a host cell with the vector into which the cleaved cDNA has
 - 30 been inserted to produce vectors containing cloned inserts;

- (e) generating linearized fragments containing the inserted cDNA molecules by digestion of the constructs produced in step (c) with at least one restriction endonuclease that does not recognize sequences in either the inserted cDNA molecules or in the bacteriophage-specific promoter, but does recognize sequences in the vector, such that the resulting linearized fragments have a 5' flanking vector sequence of at least 15 nucleotides into the vector 5' to the double-stranded cDNA molecule's second terminus;
- (f) generating a cRNA preparation of antisense cRNA transcripts by incubating the linearized fragments with a bacteriophage-specific RNA polymerase capable of initiating transcription from the bacteriophage-specific promoter;
- (g) generating first-strand cDNA by transcribing the cRNA using a reverse transcriptase and a 5' RT primer being 15 to 30 nucleotides in length and comprising a nucleotide sequence that is complementary to the 5' flanking vector sequence;
- (h) generating a first set of PCR products by dividing the first-strand cDNA into a first series of subpools and using the first-strand cDNA as templates for a first polymerase chain reaction with a first 3' PCR-primer of 15 to 30 nucleotides in length that is complementary to 3' flanking vector sequences between the first restriction endonuclease site and the site defining transcription initiation by the bacteriophage-specific promoter and a first 5' PCR-primer defined as having a 3'-terminus consisting of $-N_1$, wherein "N" is one of the four deoxyribonucleotides A, C, G, or T, the first 5' PCR-primer being 15 to 30 nucleotides in length and complementary to the 5' flanking vector sequence with the first 5' PCR-primer's complementarity extending into one nucleotide of the insert-specific nucleotides of the cRNA, wherein a different one of the first 5' PCR primers is used in each of four different subpools;
- (i) generating a second set of PCR products by further dividing the first set of PCR products in each of the first series of subpools into a second series of subpools and using the first set of PCR products as templates for a second polymerase chain reaction with a second 3' PCR primer of 15 to 30 nucleotides in length that is complementary to 3' flanking vector sequences between the first restriction endonuclease site and the site defining transcription initiation by the bacteriophage-specific promoter and a second 5' PCR primer defined as having a 3'-terminus consisting of $-N_1-N_x$, wherein N_1 is identical

to the N₁ used in the first polymerase chain reaction for that subpool, "N" is as ⁱⁿ step g, and "x" is an integer from 1 to 5, the primer being 15 to 30 nucleotides in length and complementary to the 5' flanking vector sequence with the primer's complementarity extending across into the insert-specific nucleotides of the cRNA in a number of

5 nucleotides equal to "x" + 1, wherein a different one of the second 5' PCR primers is used in different subpools of the second series of subpools and wherein there are 4^x subpools in the second series of subpools for each of the subpools in the first set of subpools; and

(j) resolving the second set of PCR products to generate a display of sequence-specific products representing the 3'-ends of mRNAs present in the mRNA
10 population.

2. The method of claim 1, wherein a biotin moiety is conjugated to the anchor primers.

3. The method of claim 2, wherein the biotin moiety is conjugated to the 5' terminus of the anchor primer.

15 4. The method of claim 2, wherein the first restricted cDNA is separated from the remainder of the cDNA in step b of claim 1 by contacting the first restricted cDNA with a streptavidin-coated substrate.

5. The method of the claim 1 wherein the 3 nucleotides at the 3' end of the first 5' PCR primer are joined by phosphorothioate linkages.

20 6. The method of the claim 1, wherein the 3 nucleotides at the 3' end of the second 5' PCR primer are joined by phosphorothioate linkages.

7. The method of the claim 1 wherein the 3 nucleotides at the 3' end of the first and second 5' PCR primers are joined by phosphorothioate linkages.

8. The method of claim 1 wherein one of the primers for the second PCR
25 reaction is conjugated to a fluorescent label.

9. The method of claim 8 wherein the fluorescent label is selected from the group consisting of

spiro(isobenzofuran-1(3H),9'-(9H)-xanthen)-3-one, 6-carboxylic acid,

3',6'-dihydroxy-6-carboxyfluorescein;

30 spiro(isobenzofuran-1(3H),9'-(9H)-xanthen)-3-one, 5-carboxylic acid, 3',6'-

- dihydroxy-5-carboxyfluorescein;
 spiro(isobenzofuran-1(3H), 9'-(9H)-xanthen)-3-one, 3',6'-dihydroxy-fluorescein;
 9-(2,5-dicarboxyphenyl)-3,6- bis(dimethylamino)-xanthylum
 6-carboxytetramethylrhodamine;
 5 3,6-diamino-9-(2-carboxyphenyl)-xanthylum;
 spiro[isobenzofuran-1(3H), 9'-xanthene]-6-carboxylic acid,5'-dichloro-3',6'-
 dihydroxy-2',7'-dimethoxy-3-oxo-;
 1H,5H,11H,15H-xantheno[2,3,4-ij:5,6,7-i'j']diquinolizin- 8-ium, -(2,4-
 disulfophenyl)-2,3,6,7,12,13,16,17-octahydro-, inner salt;
 10 6-((4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl) amino)
 hexanoic acid;
 6-((4,4-difluoro-1,3-dimethyl-5-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-
 indacene-3-propionyl)amino)hexanoic acid;
 15 6-(((4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)
 phenoxy)acetyl) amino)-hexanoic acid;
 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene-3-pentanoic acid;
 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propanoic acid;
 4,4-difluoro-5-phenyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid;
 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-
 20 propionic acid;
 4,4-difluoro-5-styryl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid;
 6-(((4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-
 yl)styryloxy)acetyl) aminohexanoic acid;
 25 6-(((4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)
 styryloxy)acetyl) aminohexanoic acid;
 9-(2,4(or 2,5)-dicarboxyphenyl)-3,6- bis(dimethylamino)- xanthylum, inner salt;
 and
 4, 7, 2', 4', 5', 7' hexachloro 6-carboxyfluorescein and 4, 7, 2', 7' tetrachloro 6-
 carboxyfluorescein.

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10. The method of claim 1 wherein the host cell is an Escherichia coli cell.

11. The method of claim 1 wherein the phasing residues in step (a) are -V-N-N.

12. The method of claim 1 wherein the phasing residues in step (a) are -V-N.

5 13. The method of claim 1 wherein the phasing residues in step (a) are -V.

14. The method of claim 1 wherein the "x" in step (i) is 3.

15. The method of claim 1 wherein the "x" in step (i) is 1.

16. The method of claim 1 wherein the phasing residues in step (a) are -V-N-N and the "x" in step (i) is 3.

10 17. The method of claim 1 wherein the phasing residues in step (a) are -V and the "x" in step (i) is 2.

18. The method of claim 1 wherein the anchor primers each have 18 T residues in the tract of T residues.

15 19. The method of claim 1 wherein the first stuffer segment of the anchor primers is 14 residues in length.

20. The method of claim 1 wherein the sequence of the first stuffer segment is G-A-A-T-T-C-A-A-C-T-G-G-A-A (SEQ ID NO: 2).

21. The method of claim 1 wherein the bacteriophage-specific promoter is selected from the group consisting of T3 promoter, T7 promoter and SP6 promoter.

20 22. The method of claim 1 wherein the bacteriophage-specific promoter is T3 promoter.

23. The method of claim 1 wherein the primer for priming of transcription of cDNA from cRNA has the sequence A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G (SEQ ID NO: 14).

25 24. The method of claim 1 wherein the vector is the plasmid pBC SK+ cleaved with ClaI and NotI and the 3' PCR primer in steps (h) and (i) is G-A-G-C-T-C-C-A-C-C-G-C-G-G-T (SEQ ID NO: 47).

25. The method of claim 1 wherein the vector is the plasmid pBC SK+ cleaved with ClaI and NotI and the 3' PCR primer in steps (h) and (i) is G-A-G-C-T-C-G-T-T-T-T-C-C-C-A-G (SEQ ID NO: 48).

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26. The method of claim 1 wherein the second restriction endonuclease recognizing a four-nucleotide sequence is MspI.

27. The method of claim 1 wherein the second restriction endonuclease recognizing a four-nucleotide sequence is selected from the group consisting of MboI,
5 DpnII, Sau3AI, Tsp509I, HpaII, BfaI, Csp6I, MseI, HhaI, NlaIII, TaqI, MspI, MaeII,
Sau3AI, BglII and HinPII.

28. The method of claim 1 wherein the first restriction endonuclease that recognizes more than six bases is selected from the group consisting of AscI, BaeI, FseI,
NotI, PacI, PmeI, PpuMI, RsrII, SapI, SexAI, SfiI, SgfI, SgrAI, SrfI, Sse8387I and SwaI.
10 29. The method of claim 1 wherein the first restriction endonuclease that recognizes more than six bases is NotI.

30. The method of claim 1 wherein the restriction endonuclease used in step (e) has a nucleotide sequence recognition that includes the four-nucleotide sequence of the second restriction endonuclease used in step (b).

15 31. The method of claim 30 wherein the second restriction endonuclease is MspI and the restriction endonuclease used in step (e) is Sma I.

32. The method of claim 30 wherein the second restriction endonuclease is TaqI and the restriction endonuclease used in step (e) is XhoI.

33. The method of claim 30 wherein the second restriction endonuclease is
20 HinPII and the restriction endonuclease used in step (e) is NarI.

34. The method of claim 30 wherein the second restriction endonuclease is MaeII and the restriction endonuclease used in step (e) is AatII.

35. The method of claim 30 wherein the second restriction endonuclease is Sau3AI and the restriction endonuclease used in step (e) is BglII.

25 36. The method of claim 30 wherein the second restriction endonuclease is NlaIII and the restriction endonuclease used in step (e) is NcoI.

37. A vector suitable for the practice of the method of claim 1 wherein the vector of step (c) is in the form of a circular DNA molecule having first and second vector restriction endonuclease sites flanking a vector stuffer sequence, and further
30 comprising the step of digesting the vector with restriction endonucleases that cleave the

vector at the first and second vector restriction endonuclease sites.

38. The vector of claim 37 wherein the vector stuffer sequence includes an internal vector stuffer restriction endonuclease site between the first and second vector restriction endonuclease sites.

5 39. The vector of claim 38 wherein the step (e) includes digestion of the vector with a restriction endonuclease which cleaves the vector at the internal vector stuffer restriction endonuclease site.

40. A vector chosen from the group consisting of plasmids pBC SK⁺ /DGT1, pBS SK⁺ /DGT2, pBS SK⁺ /DGT3, pBC SK⁺ /DGT4 and pBS SK⁺ /DGT5.

10 41. A vector comprising a mutiple cloning site chosen from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13.

42. The method of claim 1 wherein the mRNA population has been enriched for polyadenylated mRNA species.

15 43. The method of claim 1 wherein the resolving in step (j) of the amplified fragments is conducted by electrophoresis to display the products.

44. The method of claim 43 wherein the intensity of products displayed after electrophoresis is about proportional to the abundances of the mRNAs corresponding to the products in the original mixture.

20 45. The method of claim 43 further comprising a step of determining the relative abundance of each mRNA in the original mixture from the intensity of the product corresponding to that mRNA after electrophoresis.

25 46. The method of claim 43 wherein the step of resolving the polymerase chain reaction amplified fragments by electrophoresis comprises electrophoresis of the fragments on at least two gels.

47. The method of claim ⁴³40 further comprising the steps of:

(k) eluting at least one cDNA corresponding to a mRNA from an electropherogram in which bands representing the 3'-ends of mRNAs present in the sample are displayed;

30 (l) amplifying the eluted cDNA in a polymerase chain reaction;

- (m) cloning the amplified cDNA into a plasmid;
- (n) producing DNA corresponding to the cloned DNA from the plasmid; and
- (o) sequencing the cloned cDNA.

48. An improved method for simultaneous sequence-specific identification of
 5 mRNAs in a mRNA population comprising the steps of:
- (a) isolating an mRNA population;
 - (b) preparing a double-stranded cDNA population from the mRNA population
 using a mixture of anchor primers, each anchor primer having a 5' terminus and a 3'
 terminus and including: (i) a tract of from 7 to 40 T residues; (ii) a site for cleavage by a
 10 first restriction endonuclease that recognizes eight bases, the site for cleavage being
 located towards the 5'-terminus relative to the tract of T residues; (iii) a first stuffer
 segment of from 4 to 40 nucleotides, the first stuffer segment being located towards the
 5'-terminus relative to the site for cleavage by the first restriction endonuclease; (iv) a
 15 second stuffer segment interposed between the site for cleavage by a first restriction
 endonuclease that recognizes more than six bases and the tract of T residues, and (v)
 phasing residues defined by one of -V, -V-N, or -V-N-N located at the 3' terminus of each
 of the anchor primers, wherein V is a deoxyribonucleotide selected from the group
 consisting of A, C, and G; and N is a deoxyribonucleotide selected from the group
 consisting of A, C, G, and T, the mixture including anchor primers containing all
 20 possibilities for V and N;
 - (c) cleaving the double-stranded cDNA population with the first restriction
 endonuclease and a second restriction endonuclease, the second restriction endonuclease
 recognizing a four-nucleotide sequence, to form a population of double-stranded cDNA
 molecules having first and second termini, respectively;
 - 25 (d) inserting each double-stranded cDNA molecule from step (b) into a vector
 in an orientation that is antisense with respect to a T3 promoter within the vector to form
 a population of constructs containing the inserted cDNA molecules, thereby defining 5'
 and 3' flanking vector sequences adjacent to the 5' terminus of the sense strand of the
 inserted cDNA and the 3' terminus of the sense strand respectively, and said constructs
 30 having a 3' flanking vector sequence at least 15 nucleotides in length between said first

restriction endonuclease site and a site defining transcription initiation in said promoter;

(e) transforming Escherichia coli with the vector into which the cleaved cDNA has been inserted to produce vectors containing cloned inserts;

(f) generating linearized fragments containing the inserted cDNA molecules
5 by digestion of the constructs produced in step (c) with at least one restriction endonuclease that does not recognize sequences in either the inserted cDNA molecules or in the T3 promoter;

(g) generating a cRNA preparation of antisense cRNA transcripts by
10 incubating the linearized fragments with a T3 RNA polymerase capable of initiating transcription from the T3 promoter;

(h) generating first-strand cDNA by transcribing the cRNA using a reverse transcriptase and a 5' RT primer being 15 to 30 nucleotides in length and comprising a nucleotide sequence that is complementary to the 5' flanking vector sequence;

(i) generating a first set of PCR products by dividing the first-strand cDNA
15 into a first series of subpools and using the first-strand cDNA as templates for a first polymerase chain reaction with a first 3' PCR-primer of 15 to 30 nucleotides in length that is complementary to 3' flanking vector sequences between the first restriction endonuclease site and the site defining transcription initiation by the T3-specific promoter and a first 5' PCR-primer defined as having a 3'-terminus consisting of $-N_1$, wherein "N" is one of the four deoxyribonucleotides A, C, G, or T, the first 5' PCR-primer being 15 to
20 30 nucleotides in length and complementary to the 5' flanking vector sequence with the first 5' PCR-primer's complementarity extending into one nucleotide of the insert-specific nucleotides of the cRNA, wherein a different one of the first 5' PCR primers is used in each of four different subpools;

(j) generating a second set of PCR products by further dividing the first set of
25 PCR products in each of the first series of subpools into a second series of subpools and using the first set of PCR products as templates for a second polymerase chain reaction with a second 3' PCR primer of 15 to 30 nucleotides in length that is complementary to 3' flanking vector sequences between the first restriction endonuclease site and the site
30 defining transcription initiation by the T3-specific promoter and a second 5' PCR primer

defined as having a 3'-terminus consisting of $-N_1-N_x$, wherein N_1 is identical to the N_1 used in the first polymerase chain reaction for that subpool, "N" is as in step g, and "x" is an integer selected from the group consisting of 3 and 4, the primer being 15 to 30 nucleotides in length and complementary to the 5' flanking vector sequence with the primer's complementarity extending across into the insert-specific nucleotides of the cRNA in a number of nucleotides equal to "x" + 1, wherein a different one of the second 5' PCR primers is used in different subpools of the second series of subpools and wherein there are 4^x subpools in the second series of subpools for each of the subpools.

10 (k) resolving the second set of PCR products to generate a display of sequence-specific products representing the 3'-ends of mRNAs present in the mRNA population.

49. The method of claim 48 wherein the mixture of 48 anchor primers have the sequence A-A-C-T-G-G-A-A-G-A-A-T-T-C-G-C-G-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N (SEQ ID NO: 5).

50. The method of claim 48 wherein the mixture of 48 anchor primers have the sequence G-A-A-T-T-C-A-A-C-T-G-G-A-A-G-C-G-G-C-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N (SEQ ID NO: 8).

51. The method of claim 48, wherein the mixture of 12 anchor primers have the sequence A-A-C-T-G-G-A-A-G-A-A-T-T-C-G-C-G-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N (SEQ ID NO: 4).

52. The method of claim 48 wherein the mixture of 12 anchor primers have the sequence G-A-A-T-T-C-A-A-C-T-G[✓]-G-A-A-G-C-G-G-C-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N (SEQ ID NO: 7).

53. The method of claim 48 wherein the mixture of 3 anchor primers have the sequence A-A-C-T-G-G-A-A-G-A-A-T-T-C-G-C-G-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V (SEQ ID NO: 3).

54. The method of claim 48 wherein the mixture of 3 anchor primers have the sequence G-A-A-T-T-C-A-A-C-T-G-G-A-A-G-C-G-G-C-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V (SEQ ID NO: 6).

55. The method of claim 48 wherein the first restriction endonuclease is MspI and the second restriction endonuclease is NotI.

56. The method of claim 48 wherein the first 5' PCR-primer is G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N (SEQ ID NO: 22).

5 57. The method of claim 48 wherein the first 3' PCR primer and the second 3' PCR-primer are G-A-G-C-T-C-C-A-C-C-G-C-G-G-T (SEQ ID NO: 47).

58. The method of claim 48 wherein the "x" in step (j) is 3.

59. The method of claim 48 wherein the "x" in step (j) is 4.

60. A method for detecting a change in the pattern of mRNA expression in a
10 tissue associated with a physiological or pathological change comprising the steps of.

(a) obtaining a first sample of a tissue that is not subject to the physiological or pathological change;

(b) isolating an mRNA population from the first sample;

(c) determining the pattern of mRNA expression in the first sample of the
15 tissue by performing steps (a)-(j) of claim 1 to generate a first display of sequence-specific products representing the 3'-ends of mRNAs present in the first sample;

(d) obtaining a second sample of a tissue that has been subject to the physiological or pathological change;

(e) isolating an mRNA population from the second sample;

20 (f) determining the pattern of mRNA expression in the second sample of the tissue by performing steps (a)-(j) of claim 1 to generate a second display of sequence-specific products representing the 3'-ends of mRNAs present in the second sample; and

(g) comparing the first and second displays to determine the effect of the physiological or pathological change on the pattern of mRNA expression in the tissue.

25 61. The method of claim 60 wherein the physiological or pathological change is selected from the processes mediated by transcription factors, intracellular second messengers, hormones, neurotransmitters, growth factors, neuromodulators, cell-cell contact, cell-substrate contact, cell-extracellular matrix contact and contact between cell membranes and cytoskeleton.

30 62. The method of claim 60 wherein the tissue is derived from the central

nervous system.

63. The method of claim 62 wherein the physiological or pathological change is selected from the group consisting of Alzheimer's disease, parkinsonism, ischemia, alcohol addiction, drug addiction, schizophrenia, amyotrophic lateral sclerosis, multiple sclerosis, depression, and bipolar manic-depressive disorder.

64. The method of claim 62 wherein the physiological or pathological change is associated with learning or memory, emotion, glutamate neurotoxicity, feeding behavior, olfaction, vision, movement disorders, viral infection, electroshock therapy, or the administration of a drug or toxin.

65. The method of claim 60 wherein the physiological or pathological change is selected from the group consisting of circadian variation, aging, and long term potentiation.

66. The method of claim 60 wherein the tissue is derived from a structure within the central nervous system selected from the group consisting of retina, cerebral cortex, olfactory bulb, thalamus, hypothalamus, anterior pituitary, posterior pituitary, hippocampus, nucleus accumbens, amygdala, striatum, cerebellum, brain stem, suprachiasmatic nucleus, and spinal cord.

67. The method of claim 60 wherein the tissue is normal or neoplastic tissue from an organ or organ system selected from the group consisting of the cardiovascular system, the pulmonary system, the digestive system, the peripheral nervous system, the liver, the kidney, skeletal muscle, and the reproductive system.

68. The method of claim 60 wherein the tissue is normal or neoplastic tissue that comprises cells taken or derived from an organ or organ system selected from the group consisting of the cardiovascular system, the lymphatic system, the respiratory system, the digestive system, the peripheral nervous system, the central nervous system, the enteric nervous system, the endocrine system, the integument (including skin, hair and nails), the skeletal system (including bone and muscle), the urinary system and the reproductive system.

69. The method of claim 60 wherein the tissue is normal or neoplastic tissue that comprises cells taken or derived from the group consisting of epithelia, endothelia,

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mucosa, glands, blood, lymph, connective tissue, cartilage, bone, smooth muscle, skeletal muscle, cardiac muscle, neurons, glial cells, spleen, thymus, pituitary, thyroid, parathyroid, adrenal cortex, adrenal medulla, pineal, skin, hair, nails, teeth, liver, pancreas, lung, kidney, bladder, ureter, breast, ovary, uterus, vagina, testes, prostate, penis, eye and ear.

70. A method of detecting a difference in action of a drug to be screened and a known compound comprising the steps of-

- (a) obtaining a first sample of tissue from an organism treated with a compound of known physiological function;
- 10 (b) isolating an mRNA population from the first sample;
- (c) determining the pattern of mRNA expression in the first sample of the tissue by performing steps (a)-(j) of claim 1 to generate a first display of sequence-specific products representing the 3'-ends of mRNAs present in the first sample;
- (d) obtaining a second sample of tissue from an organism treated with a drug to
15 be screened for a difference in action of the drug and the known compound;
- (e) isolating an mRNA population from the first sample;
- (f) determining the pattern of mRNA expression in the second sample of the tissue by performing steps (a)-(j) of claim 1 to generate a second display of sequence-specific products representing the 3'-ends of mRNAs present in the second sample; and
- 20 (g) comparing the first and second displays in order to detect the presence of mRNA species whose expression is not affected by the known compound but is affected by the drug to be screened, thereby indicating a difference in action of the drug to be screened and the known compound.

71. The method of claim 70 wherein the drug to be screened is selected from the
25 group consisting of antidepressants, neuroleptics, tranquilizers, anticonvulsants, monoamine oxidase inhibitors, and stimulants.

72. The method of claim 70 wherein the drug to be screened is selected from the group consisting of anti-parkinsonism agents, skeletal muscle relaxants, analgesics, local anesthetics, cholinergics, antiviral agents, antispasmodics, steroids, and non-steroidal anti-
30 inflammatory drugs.

73. A database comprising the data produced by the quantitation of the display of sequence-specific products of claim 1.

74. The database of claim ²³1, further comprising data concerning sequence relationships, gene mapping and cellular distributions.

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